^{1}H-NMR studies of the histidine residues of human choriogonadotropin and its α- and β-subunits

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The pH-dependence of the chemical shifts of the imidazole C2-proton resonances of the 4 histidine residues of human choriogonadotropin has been determined in the intact hormone and in its isolated α- and β-subunits. The single histidine of the β-subunit and two of the histidines of the α-subunit show only minor changes in pK when the subunits recombine. However, one histidine of the α-subunit, tentatively identified as Hisα83, has a very low pK (≤ 2.4) in the isolated subunit and increases markedly on recombination with the β-subunit, strongly suggesting that a conformational change occurs. This behaviour is closely similar to that reported earlier for porcine lutropin.

Choriogonadotropin *Nuclear Magnetic Resonance* (NMR) *Histidine* *Subunit interactions* *Glycoprotein hormones*

1. INTRODUCTION

Choriogonadotropin (CG) is synthesised in large amounts by the placenta during the first weeks of pregnancy, and acts to stimulate the corpus luteum. Like the pituitary glycoprotein hormones lutropin, follitropin and thyrotropin, CG is composed of two non-identical subunits, α and β. The α-subunits of these 4 hormones are almost identical within a given species, while the β-subunits differ both in amino-acid sequence and in oligosaccharide structure, thus conferring hormonal specificity [1,2]. The principal biological activities of CG resemble those of lutropin, but it also shows thyrotropin- and follitropin-like activities [3,4], so that the hormonal specificity of CG is less strictly defined than that of the pituitary glycoprotein hormones.

Several experiments indicate that both subunits of these hormones possess binding sites for the target cell receptors [5–7]. In this context, the fact that the α-subunit is common to all 4 glycoprotein hormones raises some interesting questions. Do the receptors for each of the 4 hormones have a structurally homologous region which interacts with a conformationally identical α-subunit in each case, or does the interaction with the β-subunit alter the conformation of the α-subunit in a hormone-specific way? In a ^{1}H-NMR study of porcine lutropin [6], we demonstrated that the interaction between the subunits leads to a conformational change in the carboxy-terminal part of the α-chain, including His α87, Tyr α92 and Tyr α93. Furthermore, we concluded that residues α92 and α93 interact directly with testis receptors [6]. We describe here ^{1}H-NMR studies of subunit interactions in CG, designed to establish whether this change in conformation of the α-subunit is hormone-specific.

2. MATERIALS AND METHODS

Human choriogonadotropin (10000 IU/mg) and its separate α- and β-subunits were obtained from UCB Bioproducts (Brussels). Cross contamination of one subunit by the other was <2%.

The amide protons of the α- and β-subunits were replaced by deuterium by incubation in ^2H_2O (98.5

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atom %) containing 50 mM potassium phosphate (pH* 7.8) followed by lyophilisation. The notation pH* indicates a pH meter reading uncorrected for the isotope effect on the glass electrode. In order to exchange the amide protons of intact CG, a hormone solution (1%, w/v, in ^2H_2O) was adjusted to pH* 2 with ^2HCl and incubated overnight at room temperature to dissociate the subunits. The solution was then adjusted to pH* 7.8 with K_2HPO_4 and incubated a further 24 h to permit reassociation of the subunits before lyophilisation.

The imidazole C2-proton of the single histidine of the β-subunit was exchanged for deuterium by incubating a solution of β-subunit (2 mM in ^2H_2O containing 50 mM potassium phosphate, 0.1% azide, pH* 7.8) for one week at 40°C. After lyophilisation, the ^2H-exchanged β-subunit was recombined with a stoichiometric amount of α-subunit (amide protons exchanged as described above) by incubation of a 1% protein solution in ^2H_2O containing 100 mM potassium phosphate, 0.02% azide, pH* 7.8 for 24 h at 37°C. All protein samples were lyophilised and redissolved in ^2H_2O for the NMR experiments; final protein concentrations were 30 mg/ml for the isolated subunits and 60 mg/ml for the recombined hormone.

270 MHz ^2H-NMR spectra were obtained using a Bruker WH270 spectrometer. All spectra were recorded at a sample temperature of 40°C, and pH adjustment during titration experiments was also carried out at this temperature, by addition of microlitre volumes of 0.5 M NaO^2H or ^2HCl in ^2H_2O. A spectral width of 4.2 kHz was used, with quadrature detection; 1500–2000 transients were averaged with a pulse interval of 0.5 s. When the water proton resonance became too large, it was minimised by saturation with a selective 0.4 s pulse immediately before the non-selective observing pulse. Dioxan was present in all samples at 2 mM as a chemical shift reference.

After the NMR experiments, the protein samples were desalted on Sephadex G25 in 50 mM ammonium bicarbonate buffer and lyophilised. The integrity of the recovered proteins was checked by radioimmunoassay systems specific for human CG and for its α- and β-subunits and by the binding of recombined CG to particulate receptor from immature porcine testis [8], which was assayed by competition with ^{125}I[CG]. After the NMR experiments, the α- and β-subunits proved to be as immuno-reactive as before the deuterium exchange, while the recombined CG showed 60% (receptor-binding assay) to 75% (radioimmunoassay) of the activity of native CG.

3. RESULTS AND DISCUSSION

Both CG [9–12] and porcine lutropin [13,14] contain 4 histidine residues, 3 in the α-subunit and 1 in the β-subunit; those in the α-subunit, but not that in the β-subunit, occupy homologous positions in the two sequences.

In the ^1H-NMR spectrum of the isolated CG α-subunit 3 C2-H resonances from the 3 histidine residues could be observed; the pH-dependence of their chemical shifts is shown in fig.1. Two of the histidine residues, A and B, show more or less normal titration curves, with pK values and chemical shifts (table 1) in the expected range. (For CG, as noted earlier for lutropin [6], exchange broadening of the C2-H resonances limits the precision with which the pK values can be determined to $\pm$ 0.1 unit.) The third histidine residue shows an unusual chemical shift for its C2-H resonance in the unprotonated form (δ_A = 4.29 ppm), and an abnormally low pK ($\leq$ 2.4; the resonance broadens markedly below pH* 2.6). Very similar behaviour (δ_A = 4.28 ppm, pK = 3.3) was noted for His α87 of porcine lutropin [6], strongly suggesting that the 'abnormal' histidine residue in the human CG α-

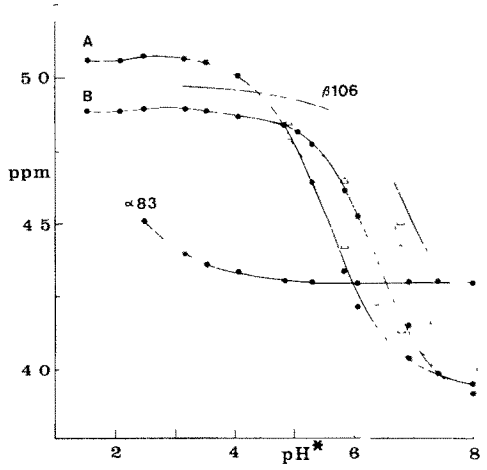

Fig.1. pH-dependence of the chemical shifts of the imidazole C2-proton resonances of intact human choriogonadotropin (Δ—Δ), its isolated α-subunit (●—●) and its β-subunit (○—○).

Table 1

pK-Values and C2-H chemical shifts of the histidine residues of the isolated α- β-subunits of human choriogonadotropin and porcine lutropin

	pK	δ_{HA+} [a]	δ_{A-} [a]
Choriogonadotropin			
His A[b]	5.7 (± 0.1)	5.07	(3.9)[e]
His B[b]	6.4 (± 0.1)	4.89	(3.85)[e]
His α83	≤2.4	[d]	4.29
His β106	7.2 (± 0.1)	4.98	(3.9)[e]
Lutropin[c]			
His α83	5.4 (± 0.1)	4.97	3.96
His α87	3.3 (± 0.05)	4.84	4.28
His α94	6.0 (± 0.1)	4.86	3.88
His β89	5.6 (± 0.1)	5.01	3.97

[a] Chemical shifts in the protonated (δ_{HA+}) and unprotonated (δ_A) forms of the imidazole
[b] Histidines of the α-subunit not individually assigned
[c] From [6]
[d] Not determined
[e] Assumed for calculation of pK

subunit is the homologous residue His α83. In lutropin α-subunit, protonation of His α87 was clearly associated with a significant conformational change [6]. The observation of small changes in chemical shift of the C2-H resonances of histidines A and B at about pH* 2.4 suggests that this may also be the case in CG. If this conformational change must occur before His α83 can be protonated, then a difference in the stability of the protein towards this pH-induced partial unfolding could account for the difference in pK between the homologous His α83 (CG) and His α87 (lutropin). Certainly the virtually identical, unusual C2-H chemical shifts of the unprotonated form of these residues argues that their immediate environment is closely similar in the two proteins.

The single histidine residue of the β-subunit, His β106, also shows a normal titration curve (fig.1); measurements were not made above pH* 7.4, but assuming δ_A = 3.9 ppm, this residue has a pK of ~7.2 (table 1).

In intact CG, a number of amide NH resonances persist in the spectrum, in spite of the exchange procedures described in section 2. Together with the exchange broadening of the C2-H resonances noted above, this gives rise to some difficulty in resolving individual C2-H resonances. At high pH, only two C2-H peaks were resolved; the lower field peak had the greater intensity, and as the pH was decreased it split into two components. Below pH* 5, the hormone begins to dissociate into its subunits, thus limiting the pH-range over which measurements could be made. The partial titration curves obtained are shown by the dashed curves in fig.1.

With a total of 4 histidine residues in the protein, the higher field (less intense) resonance must correspond to a single histidine residue with a pK of ~5.7. The lower field peak then contains the resonances of 3 histidine residues. Comparison of the spectrum of normal CG with that of a sample of CG, reconstituted with β-subunit in which the imidazole C2-proton had been exchanged for deuterium, showed that one of these 3 histidines is His β106, whose pK had thus decreased by ~0.4 units on interaction with the α-subunit.

Since the histidines of the α-subunit have not been individually identified in the spectrum of intact CG, we can not describe the changes in their pK-values precisely. As far as histidines A and B are concerned, the simplest hypothesis is that the pK of histidine is unchanged and that that of histidine B increased by about 0.4 units on binding to the β-subunit. It is quite clear, however, that there is no resonance in the spectrum of the intact hormone corresponding to that of the 'abnormal' histidine of the α-subunit, His α83. The pK of this residue must have increased by at least 3.3 units on interaction with the β-subunit to form the whole hormone.

A similarly large increase in pK on recombination with the β-subunit was observed for the homologous His α87 of porcine lutropin [6]. The direction of the change, from an abnormal towards a normal pK, is such that it cannot arise simply from burying this histidine residue in the subunit interface, but must reflect a change in the conformation of the α-subunit on combination with the β-subunit. The change in environment of this histidine residue of the α-subunit appears to be very similar, whether the β-subunit with which it combines is that of CG or lutropin. At least as far as this residue is concerned, the change in conformation of the α-subunit is not hormone-specific. In a study of glycoprotein hormones [15] the same conclusion was reached.

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REFERENCES

[1] Pierce, J.G., Faith, M.R., Giudice, L.D. and Reeve, J.R. (1976) in: Polypeptide Hormones: Molecular and Cellular Aspects. Ciba Foundation Symposium 41, pp. 225–265, Elsevier/Excerpta Medica, Amsterdam, New York.

[2] Maghuin-Rogister, G., Closset, J. and Hennen, G. (1976) in: 'Peptides 1976', Proc. 14th European Peptide Symposium (Loffet, A. ed) pp. 325–338, Editions de l'Université de Bruxelles.

[3] Taliadouros, G.S., Canfield, R.E. and Nisula, B.C. (1978) Endocrinology 47, 855–860.

[4] Siris, E.S., Nisula, B.C., Catt, K.J., Horner, K., Birken, S., Canfield, R.E. and Ross, G.T. (1978) Endocrinology 102, 1356–1361.

[5] Parsons, T.F. and Pierce, J.G. (1979) J. Biol. Chem. 254, 6010–6015.

[6] Maghuin-Rogister, G., Degelaen, J. and Roberts, G.C.K. (1979) Eur. J. Biochem. 96, 59–68.

[7] Moyle, W.R., Ehrlich, P.H. and Canfield, R.E. (1982) Proc. Natl. Acad. Sci. USA 79, 2245–2249.

[8] Maghuin-Rogister, G., Closset, J., Combarnous, Y., Dechenne, Ch., Hennen, G. and Ketelslegers, J.M. (1978) Eur. J. Biochem. 86, 121–131.

[9] Bellisario, R., Carlsen, R.B. and Bahl, O.P. (1973) J. Biol. Chem. 248, 6796–6809.

[10] Carlsen, R.B., Bahl, O.P. and Swaminathan, N. (1973) J. Biol. Chem. 248, 6810–6827.

[11] Kessler, M.J., Mise, T., Ghai, R.D. and Bahl, O.P. (1979) J. Biol. Chem. 254, 7909–7914.

[12] Morgan, F.J., Birken, S. and Canfield, R.E. (1975) J. Biol. Chem. 250, 5247–5258.

[13] Maghuin-Rogister, G., Combarnous, Y. and Hennen, G. (1973) Eur. J. Biochem. 39, 255–263.

[14] Maghuin-Rogister, G. and Hennen, G. (1973) Eur. J. Biochem. 39, 235–254.

[15] Parsons, T.F. and Pierce, J.G. (1983) Int. J. Protein Peptide Chem. in press.